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ORIGINAL RESEARCH

Clinical Impact of Molecular Testing for Respiratory Viruses in Children Admitted with Acute Respiratory Diseases: Real-World Evidence

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Aim: To describe the impact of molecular testing for respiratory viruses (MTRV) integrated in an antimicrobial and diagnostic stewardship program analyzing antimicrobial prescription and length of stay (LOS) of children hospitalized with acute respiratory disease in a resource-limited setting.

Methods: A retrospective case–control study was designed involving children hospitalized with acute respiratory disease. Interventions in the case group included implementation of MTRV in a 24 h clinical microbiology service, results available during the first day of hospitalization, training technicians and medical staff in sampling, results interpretation, supervisions and recommendation about antibiotic prescriptions by pediatric infectious diseases and pneumologist. Main outcomes were mean LOS, antibiotic prescriptions and days of therapy (DOT).

Results: A total of 1200 children were included (396 cases and 804 controls), most of whom were younger than 5 years. Median LOS was shorter in the case group (5 vs 6 days; $p \leq 0.05$), with a 17.1% increase in hospitalizations lasting 1–4 days and a reduction in stays of 5–10 days ($p<0.001$). Antibiotic prescription was significantly lower in cases from day 1 (34.5% vs 81.0%; $p<0.001$) and remained lower on days 3 and 5. Viral detection on the first hospital day was associated with up to a 20% reduction in antibiotic use. We observed a reduction in DOTs/100 days for aminopenicillins with beta-lactamase inhibitors, third-generation cephalosporins, and macrolides ($p<0.001$). Viruses were detected in 66.4% of cases, mainly rhinovirus/enterovirus, RSV, SARS-CoV-2, and parainfluenza virus type 3.

Conclusion: The integration of MTRV testing into a stewardship program reduced antibiotic use and shortened hospital stays in children with acute respiratory disease. Supporting the value of rapid molecular diagnostics as a key component of pediatric antimicrobial stewardship, especially in resource-limited settings.

Keywords: acute respiratory disease, molecular testing for respiratory viruses, antimicrobial stewardship programs

Introduction

Acute respiratory diseases (ARDs) represent a major cause of morbidity and mortality in children under 5 years of age. However, although viral pathogens account for a large proportion of cases, more than 70% of patients are unnecessarily treated with antibiotics.[1,2] Consistent with global trends, Ecuador experienced high mortality during the COVID-19 pandemic.[3] Hospital General IESS Sur de Quito, a key reference center during the pandemic, subsequently faced a high burden of pediatric care. According to the hospital's statistics department, pediatric visits for ARDs increased markedly, from approximately 9365 cases in 2021 to 36,251 cases in 2022. Concomitantly, unnecessary antibiotic prescribing represents an

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important concern, as it directly increases healthcare costs; our analysis estimates the cost of each pediatric hospitalization day to be approximately USD 250–300.

In recent years, antimicrobial stewardship programs (ASP) have proven to be strategies capable of successfully mitigating antibiotic overuse, decreasing rates of antibiotic resistance, length of hospital stay, antibiotic-associated adverse drug events, hospital readmission, and mortality.[4] Additionally, the implementation of rapid molecular diagnostic tests for the detection of multiple pathogens has been shown to support ASP, primarily by reducing hospital length of stay (LOS) and antibiotic prescriptions at discharge.[5] However, the high cost of these tests and the need for careful interpretation of results are important considerations for clinical decision-making, particularly in resource-limited settings. Therefore, although their use may be beneficial, the integration of diagnostic stewardship programs (DSP) in hospital settings should be considered essential.[6]

This study evaluates the impact of an ASP integrated with a DSP using a Multiplex viral molecular panel based on reverse transcription–polymerase chain reaction (MTVPR) on antibiotic prescribing and mean LOS in children hospitalized with ARD.

Study Population and Design

A retrospective case–control study was developed in the Hospital General IESS Sur de Quito belongs to the Social Security Institute of Ecuador (IESS, in Spanish) with 400 beds acting as reference spot to COVID-19-like illness surveillance in adult and children since the start of the pandemic in 2020.[6] The study involved to children aged 1 to 18 years hospitalized with ARD diagnosis. Cases and controls were identified using two distinct databases. Cases were defined based on MTVPR results and hospitalized between January and December 2021 where the collaborative ASP-DSP strategy was implemented, whereas controls were identified according to the diagnosis recorded at hospital admission before the implementation of the ASP-DSP intervention. Both groups met predefined inclusion criteria and were matched in a 2:1 ratio by the institutional statistics department. In compliance with the Declaration of Helsinki, an anonymized database was created by the statistical department of the Hospital General IESS Sur de Quito with the approval of the (CEISH), using data from electronic health records (EHRs). The analysis was performed exclusively on this dataset, ensuring that patient re-identification was not possible.

The Collaborative ASP-DSP Intervention in Cases Group

For cases, the ASP-DSP Interventions were performed in two main settings of the hospital. First, activities in the microbiology laboratory included: training in implementation, interpretation, and release of molecular results within 2–4 h once nasopharyngeal samples were received, and the permanent availability of the clinical microbiology service, ie, a 24-h setting.

Second, activities at hospitalization wards included education to medical doctor's staff (ie, general physicians, pediatricians) to prescribe MTRV of nasopharyngeal samples, sampling techniques, results interpretation, and antibiotic supervision prescriptions, especially prescriptions made by pediatric infectious disease and pneumologist specialists.

Two MTRV panels were available in the hospital and were used over nasopharyngeal or throat swab samples depending on the practicality of collection. First, the BioFire® FilmArray® Respiratory Panels RP2.1 V (BioFire Diagnostics, Salt Lake City, UT, USA), detecting Adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus OC43, coronavirus NL63, SARS-CoV-2, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A/H1, influenza A/H1-2009, influenza A/H3, influenza B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, and respiratory syncytial virus (RSV). Second, the ePlex Respiratory Pathogen (RP) panel (GenMark Diagnostics) for detection of adenovirus, human bocavirus, coronavirus species (229E, HKU1, OC43, NL63, and SARS CoV-2), influenza B, influenza A, influenza A H1, influenza A H1-2009, influenza A H3, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, respiratory syncytial virus A, respiratory syncytial virus B, human metapneumovirus, and human rhinovirus/enterovirus.

Data Collection and Main Outcomes

The primary outcomes were LOS and antibiotic prescription; all data were extracted from an anonymized database derived from electronic health records (EHR) provided by the Hospital's statistical department. For LOS, we grouped patients based on hospitalization days (ie 1–4, 5–10, or more than 10) to highlight intervention impact. For antibiotic prescription, we collected two variables: patients' percentage that received antibiotic therapy in each hospitalization day

and the total days of antibiotic therapy (DOT) per 100 bed-day following Mirjana et al which considers DOT as the number of days that a patient receives antibiotics regardless of the dose.[7] All doses of a specific antibiotic administered on a given day were considered as one DOT. If a patient received more than one antibiotic, then more than one DOT was counted.[7] DOTs were measured 12 h after MTVR results became available in EHR for the intervention group and 12 h after pediatric ward admission for the control group. Demographic data, key clinical dates, viral detection frequency, and other relevant variables were collected.

Statistical Analysis

Results were processed using descriptive summary statistics such as percentages and frequencies. We applied the normality test Kolgomorov–Smilnorv to all the variables. Statistical differences were assessed by χ^2 and Fisher's exact test when comparing categorical variables, and by Mann–Whitney *U*-test when comparing medians in quantitative variables.

Ethical Aspects

The present study with code 2023–031M, was approved by the Research Ethics Committee on Human Subjects (CEISH) from Universidad San Francisco de Quito-Ecuador in 2023.

Results

A total of n=1200 children were included, from them n=396 were cases and n=804 controls. Most of them were <5 years old (67.9% and 71% for cases and controls, respectively). The mean age was 3 years old for cases and 2 years old for controls. Females were most common in both groups.

The median LOS was 6 days in the control group and five days in the case group (Mann–Whitney *U*-test, $p \leq 0.05$). In the case group, hospitalizations lasting 1–4 days increased by 17.1%, with a corresponding reduction in stays of 5–10 days (Mann–Whitney *U*-test, $p < 0.001$ (Table 1).

On the first day of hospitalization, 34.5% of patients in the case group (137/396) received antibiotics compared with 81.0% in the control group (648/804) (Pearson's $\chi^2 = 58.41$, df = 2, $p < 0.001$). Antibiotic prescription decreased by day 3 to 29.0% (114/396) in the case group and 53.0% (429/804) in the control group (Pearson's $\chi^2 = 26.03$, df = 2, $p < 0.001$).

Table 1 Demographic and Clinical Date of Cases and Controls

Variables	Control n=804 (%)	Cases n=396 (%)	Pearson/Mann Witney *U*-test P value
Age in year - mean (IQR) (maximum/minimum)	2 (1–6)	3 (1–8)	
Range (years)			
<2	249 (31.0)	143 (36.1)	p≤0.05 [0.016]
2–5	332 (41.3)	126 (31.8)	
6–10	146 (18.2)	80 (20.2)	
11–15	47 (5.8)	31 (7.8)	
16–17	20 (3.7)	16 (4.0)	
Female	347 (45.4)	173 (43.7)	p≥0.05 [0.574]
Male	417 (54.6)	223 (56.3)	
Survival	804 (100)	396 (100)	

(*Continued*)

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Table 1 (Continued).

Variables	Control n=804 (%)	Cases n=396 (%)	Pearson/Mann Witney *U*-test P value
Wood-Downes-Ferres scoring system[§] (Mean maximum/minimum)			
0–3		200 (32.3)	
4–7		6 (1.0)	
8–9		1 (0.2)	
Length of stay–average (IQR), (day) mean (maximum/ minimum)	6.2 (32–1)	5.8 (46–0)	
Length of stay –mean (IQR), (day)	6 (4–7)	5 (3.25–7)	p≤0.05
1–4	211 (26.3)	172 (43.4)	p≤0.05
5–10	546 (67.9)	196 (49.5)	
≥ 10	47 (5.8)	28 (7.1)	
Antibiotic prescription (days)			
0	648 (85)	137 (34.5)	p≤0.01
3	514 (53)	114 (29)	
5	266 (33)	81 (20.4)	
Media of therapy, day	5.71	1.65	p≤0.05

Notes: Variables show the variables related to demographics: age, gender and survival rate. [§]The clinical variables including Wood-Downes-Ferres scoring system used for acute pulmonary pediatric urgencies including asthma, bronchiolitis, length of stay in the hospital, antibiotic prescription and media of days that the patient received therapy.

Finally, on the 5th day, these prescriptions were 20.4% (81/396) for the cases group and 33% (266/804) for the control group (X2 Pearson = 11.77, df=2 $p<0.001$) (Figure 1).

We observed up to 20% less antibiotics prescription in cases with virus detection on day one of hospitalization (Figure 2).

Further, we observed a decrease of DOTs/100d in cases group, specifically: 18.58 for Aminopenicilin + beta-lactamase inhibitor (BLI), 24.7 in third-generation cephalosporin (Ceftriaxone), and 18.7 in macrolides ($p<0.001$; Table 2).

Viruses were detected in 66.4% of cases (n=263/396). The most frequently were rhinovirus/enterovirus, RSV A/B, SARS-CoV-2, and parainfluenza virus type 3. SARS-CoV-2 was detected in 43 of 396 children (10%). Viral coinfections (≥2 viruses) were observed in 27 children (10.2%), most commonly RSV plus rhinovirus/enterovirus (n=4), SARS-CoV -2 plus RSV A/B (n=4), adenovirus plus rhinovirus/enterovirus (n=3), and rhinovirus/enterovirus plus parainfluenza virus type 3 (n=3); other combinations accounted for 10 cases (Table 3).

Discussion

To our knowledge, this is the first case–control study in Ecuador evaluating a collaborative ASP–DSP strategy based on MTRV in hospitalized children with ARDs. The ASP–DSP intervention was associated with reduced length of stay and days of therapy, and with lower antibiotic use during the peak year of the COVID-19 pandemic, a period characterized by widespread antibiotic overuse and major strain on healthcare systems.[9]

ASP-based interventions in children have been shown to reduce the use of broad-spectrum antibiotics. Such strategies include prospective audit and feedback, preauthorization of antimicrobials, implementation of prescribing guidelines, and targeted education of healthcare professionals.[10] In low- and middle-income countries, studies suggest that multifaceted approaches—combining educational interventions with clinical decision-support tools—are more effective than

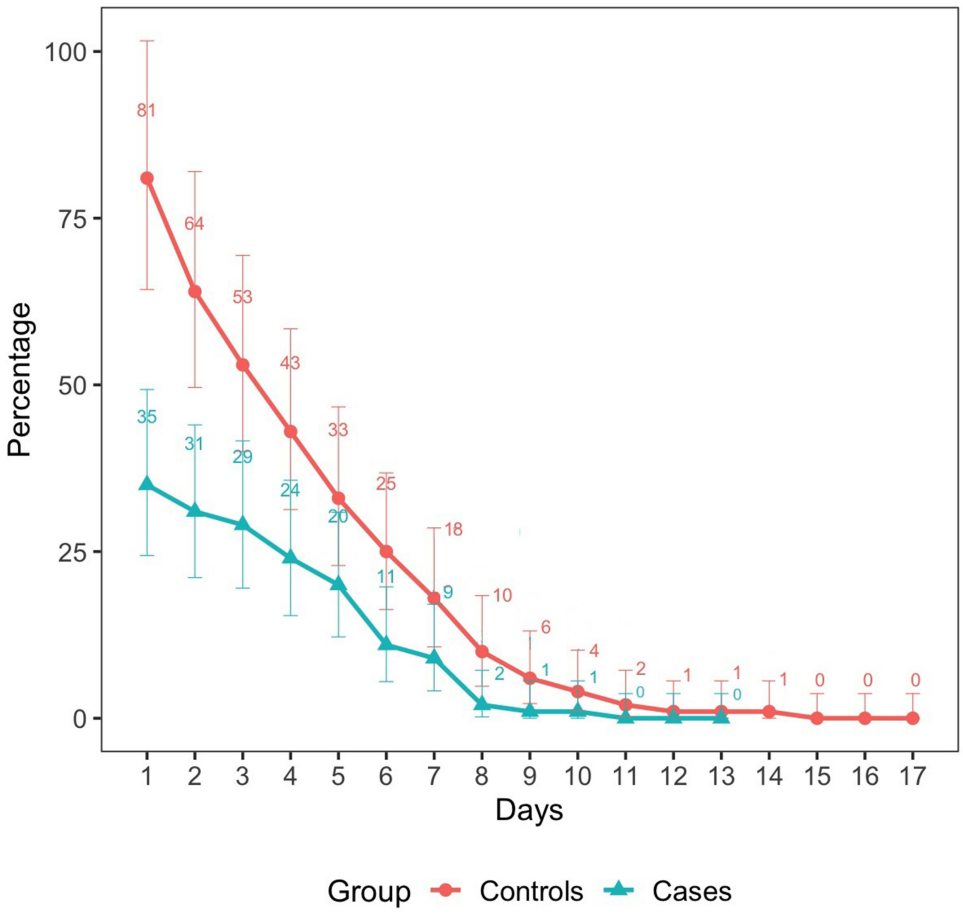

Figure 1 Percentage of antibiotic prescription in cases and controls. During the first day, up to 80% of patients in the control group received antibiotics; at the day 8th prescription decreases to 10% (blue). On the contrary, 30% of patients in the cases group received antibiotic at the first day; this percentage fall less than 10% at day 8th.

guideline-based ASPs alone. These combined strategies are particularly relevant for reducing clinical infections and colonization with multidrug-resistant organisms.[11]

In recent years, there's been a growing body of evidence showing how MTRV can influence clinical decision-making, reducing the use of intravenous antibiotics, LOS, and needing chest X-rays, inclusive early adoption of isolation precautions.[8] This effectiveness is attributed to their sensitivity and specificity, which are reported as follows: 91% and 99% for influenza A; 91% and 98% for RSV; 67% and 99% for adenovirus;[9] and 98% and 94% for SARS-CoV-2,[10] additionally, the rapid turnaround time to detect pathogens has been also implicated in these advantages. However, if a decision depended only of these attributes leaving aside an ASP, it may have a poor impact in antibiotic reduction consumption or inclusive the LOS.[11] Thus, the essence of our interventions—training of technicians and medical doctors staff on interpretation of MTRV assays results in a microbiology laboratory permanently available, plus supervision/pre-authorization of antibiotic prescription—provides a strong evidence that an ASP-DSP collaborative framework has a positive impact on antibiotic consumption and LOS in hospitalized children with ARS. The rapid microbiological information generated by molecular assays constitutes a key justification for the continuous 24-h operation of the microbiology laboratory.[12] We suggest that in hospitals where it is not feasible, the supervision or pre-authorization of antibiotic prescriptions could be a main intervention to reduce the antibiotic consumption and LOS.[13]

During COVID-19 pandemic course, several reports highlighted the negative correlation of non-pharmaceutical interventions such as facemasks, avoidance of mass gathering events, or school closures, with changes in epidemiology of respiratory-borne infections especially for Influenza virus and RSV.[14] Nevertheless, we found an increase in

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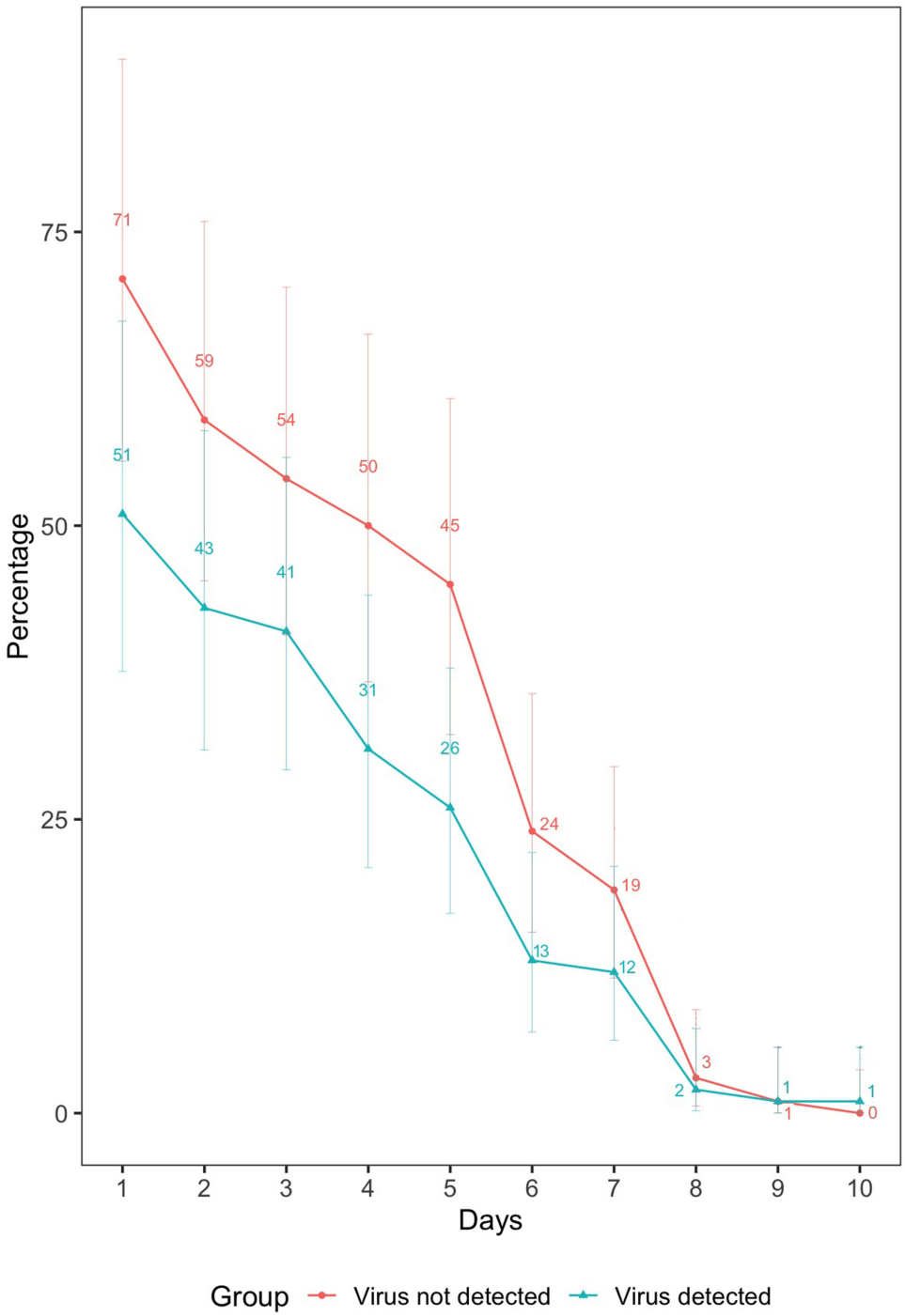

Figure 2 Percentage of cases with antibiotic prescription according to molecular assays results.

hospitalizations by Rhinovirus/Enterovirus followed by RSV in our cohort that can probably be explained by the implementation of non-pharmaceutical interventions across all Latin American countries.[15]

This study has several limitations inherent to its retrospective design. First, data were obtained from medical records, which may result in incomplete, heterogeneous, or inaccurately recorded information. It was not possible to standardize the control of relevant clinical variables, such as the indication for diagnostic studies, the initiation and duration of antibiotic therapy, or the ordering of additional tests, all of which may have influenced clinical decision-making, second, during the peak of the COVID-19 pandemic (ie, 2020), could be influenced by a larger use of antibiotic prescription

Table 2 The Table Describes DOTs Reached in Cases and Controls

Antibiotic Class	Group	DOT Total	DOT per 100 Days-Patients	IRR (Cases vs Controls)	IC 95%	P value
Aminopenicilin	Cases	154	6.70	0.55	0.46–0.66	< 0.001*
	Controls	489	10.14			
Aminopenicilin + IBL	Cases	270	11.75	0.33	0.29–0.38	< 0.001*
	Controls	1463	30.33			
Third-generation cephalosporin	Cases	122	5.31	0.15	0.12–0.18	< 0.001*
	Controls	1447	30.00			
Macrolides	Cases	51	2.22	0.09	0.07–0.12	< 0.001*
	Controls	1010	20.94			
Carbapenems	Cases	14	0.61	0.20	0.12–0.35	< 0.001*
	Controls	126	2.61			

Notes: *Statistical significative difference ($p < 0.05$). The analysis was done based on 2296.8 days-patient for cases and 4984.8 days-patient for controls.
Abbreviations: DOT, Days of therapy; IRR, Incidencia Rate Ratio; IBL, Inhibitor of Beta-Lactamasa.

Table 3 Virus Detected in N= 263 Children

Virus	Total (%)
Rhinovirus/enterovirus	103 (39)
Respiratory syncytial virus A/B	57 (22)
SARS-CoV-2	43 (17)
Parainfluenza virus 3	18 (7)
SARS-CoV-2 + respiratory syncytial virus A/B	4 (1.5)
Rhinovirus/enterovirus + respiratory syncytial virus A/B	4 (1.5)
Human metapneumovirus	4 (1.5)
Adenovirus + rhinovirus/enterovirus	3 (1.1)
Coronavirus NL63	3 (1.1)
Adenovirus	3 (1.1)
Rhinovirus/enterovirus + parainfluenza 3	3 (1.1)
SARS-CoV-2 + rhinovirus/enterovirus	2 (0.7)
Adenovirus + respiratory syncytial virus A/B	2 (0.7)
Coronavirus (229E + HKU1 + NL63 + OC43)	2 (0.7)
Adenovirus + parainfluenza virus 3	2 (0.7)
Coronavirus OC43	1 (0.4)
Human bocavirus	1 (0.4)
Parainfluenza virus 1	1 (0.4)
Parainfluenza virus 2	1 (0.4)
Parainfluenza virus 4	1 (0.4)
Respiratory syncytial virus A/B + parainfluenza virus 3	1 (0.4)
Parainfluenza 3 + human metapneumovirus	1 (0.4)
Rhinovirus/enterovirus + human Bocavirus	1 (0.4)
Rhinovirus/enterovirus + coronavirus 229E	1 (0.4)
Respiratory Syncytial Virus A/B + Coronavirus (OC43 + 229E)	1 (0.4)
Total	263 (100)

Notes: The table described all the viruses identified in the children included in the study, Rinovirus/Entrovirus, Respiratory Syncytial Virus A/B and SARS-Cov-2 represent the vast majority of cases identified.

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compared with 2021.[16] Third, we did not include data on confirmed bacterial infections, chest X-ray findings, or biomarkers (eg, procalcitonin and CRP), as these factors may have influenced clinical decision-making.

Conclusion

Implementation of molecular diagnostic approaches via MTRV in children between 1–18 years old in an ASP-DAP framework decreases antibiotic overuse by changing prescription behavior and LOS, which might directly reduce health care costs. As a first approach bridging ASP-DSP frameworks to antibiotic prescription behavior in respiratory illnesses, we provide important evidence that can be replicated in other hospitals in the country or across the region.

Data Sharing Statement

All data used is included in this paper, the original data base can be sent by request to the corresponding author jorgereyes83@gmail.com.

Ethics Approval Statement

The present study with code 2023-031M, was approved by the Research Ethics Committee on Human Subjects (CEISH) from Universidad San Francisco de Quito-Ecuador in 2023.

In compliance with the Declaration of Helsinki, an anonymized database was created by the statistical department of the Hospital General IESS Sur de Quito using data from electronic health records (EHRs). The analysis was performed exclusively on this dataset, and none of the authors knew any identity details of patients ensuring that patient re-identification was not possible, for this reason patient parental consent was not required by the CEISH.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

All the authors declare no conflict of interest.

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